# No Substantial Histopathologic Changes in *Mops condylurus* Bats Naturally Infected with Bombali Virus, Kenya

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We found similar mild perivascular inflammation in lungs of Bombali virus–positive and –negative *Mops condylurus* bats in Kenya, indicating the virus is well-tolerated. Our findings indicate *M. condylurus* bats may be a reservoir host for Bombali virus. Increased surveillance of these bats will be important to reduce potential virus spread.

Despite extensive research since the first documented human Ebola virus (EBOV) disease outbreak in 1976, animal species involved and mechanisms by which ebolaviruses spillover to humans remains enigmatic. Bats have been implicated as reservoir hosts (*1*), a suspicion supported by several types of evidence, none of which is strong alone. Evidence includes serologic and, rarely, PCR-based diagnosis of infection in wild bats; capacity for some bat species to become asymptomatically infected after laboratory inoculations; and close phylogenetic relationship between ebolaviruses and Marburg virus, which has an established fruit bat reservoir (*2–4*). Lack of specimens from naturally occurring ebolavirus-infected bats has precluded assessment of tissue pathology and virus distribution at a cellular level, which would normally provide insights into host roles and transmission mechanisms and permit comparisons and validation of experimental infection models.

We analyzed tissue specimens collected from wild bats in Kenya that were naturally infected with Bombali virus (BOMV), the sixth and most recent ebolavirus identified. First reported in *Mops condylurus* and *Chaerephon pumilus* bats in 2018 (*5*), BOMV has been detected by PCR at low prevalence (0.5%–6.7%) in excreta or tissue samples from *M. condylurus* bats in 4 distinct locations across sub-Saharan Africa (*5–8*), representing a consistent ebolavirus–bat host relationship. No further reports of infected *C. pumilus* bats or that BOMV causes human disease have been published (*6*), although prudence is warranted. All other ebolaviruses found in Africa can cause severe human disease (only 1 nonfatal infection with Taï Forest virus has been reported) (*9*). BOMV binds to the same Niemann-Pick C1 endosomal receptor for cell entry as other ebolaviruses (*5*), and recent serologic evidence of a cleared human BOMV infection has been reported (*10*).

## The Study

We captured bats by using mist nets in Busia and Taita-Taveta counties in Kenya during 2019. In brief, we captured, euthanized, and dissected bats and collected tissue samples. We screened the samples for BOMV by using reverse transcription PCR (RT-PCR) as described previously (*8*). We placed bat carcasses that had remaining tissues immediately into 10% buffered formalin, transferred them to 80% ethanol after 24–48 h, and shipped those to the University of Helsinki, Finland, for histopathologic assessment. We analyzed 3 BOMV RT-PCR–positive and 6 BOMV RT-PCR–negative *M. condylurus* bats; the BOMV-negative bats were used as negative controls (*8*).

We trimmed and processed formalin-fixed tissue samples from the 9 bats for histologic evaluation. We routinely stained tissue sections with hematoxylin and eosin. After heat-induced antigen retrieval, we also immunolabeled sections from each tissue sample

**Table.** Lesions found in bat tissues stained with hematoxylin and eosin in study of histopathologic changes in *Mops condylurus* bats naturally infected with BOMV, Kenya*

| Organ | Lesion | BOMV-infected† | Uninfected† |
|---|---|---|---|
| Lung | Mild perivascular and interstitial mononuclear infiltrates | 3/3 | 4/6 |
| | Scattered thickening of pulmonary vessel walls | 2/3 | 1/6 |
| | Mild peribronchial mononuclear infiltrates | 1/3 | 2/6 |
| Liver | Mild perivascular mononuclear infiltrates | 3/3 | 1/6 |
| | Moderate chronic neutrophilic infiltrates | 0/3 | 3/6 |
| | Mild hepatocellular vacuolation | 0/3 | 1/6 |
| Heart | Mild mononuclear infiltrates | 0/3 | 2/6 |
| Stomach | Moderate chronic granulomatous gastritis with intralesional nematodes | 0/3 | 2/6 |

*Comparisons were made between BOMV RT-PCR–positive (BOMV-infected) and RT-PCR–negative (uninfected) *Mops condylurus* bats. Lesions likely represent spontaneous background lesions, except for the neutrophilic infiltrates in liver tissue and granulomatous gastritis nematodiasis. BOMV, Bombali virus.
†Number of bats with lesions/total number of bats per group (infected or uninfected).

by using polyclonal rabbit serum against EBOV matrix protein VP40 (*11*) (Appendix, https://wwwnc.cdc.gov/EID/article/29/5/22-1336-App1.pdf). No BOMV isolates were available for this study, which necessitated finding a suitable, cross-reactive surrogate virus. EBOV is the most studied member in the ebolavirus family, and EBOV antibodies are readily available. EBOV and BOMV have ≈75% amino acid identity match within the VP40 protein (Appendix Figure); therefore, we considered EBOV VP40 to be a suitable antigen to detect BOMV VP40.

We observed mild perivascular and interstitial mononuclear infiltrates in all 3 BOMV RT-PCR–positive *M. condylurus* bat lung samples stained



**Figure 1.** Representative tissue section from the lung of a bat in study of histopathologic changes in *Mops condylurus* bats naturally infected with Bombali virus, Kenya. We stained lung tissue sections from a Bombali virus–positive bat with hematoxylin and eosin. Arrow indicates focal minimal mononuclear cell infiltrate. Original magnification ×200.

with hematoxylin and eosin (Table; Figure 1). We found similar nonspecific mononuclear infiltrates in lung tissue from 4/6 BOMV RT-PCR–negative bats. Overall, we did not observe substantial histopathologic changes in the lungs of any bats that suggested severe acute BOMV disease. We found that both BOMV RT-PCR–positive and RT-PCR–negative bats had mixed acute and chronic inflammatory lesions in other internal organs. Those lesions likely reflect natural disease in wild bats, and we interpreted those findings as incidental.

We did not find an association between immunologic detection of EBOV VP40 antigen, used as a surrogate for BOMV, and overt histopathologic findings in BOMV RT-PCR–positive *M. condylurus* bats. We previously showed that BOMV RT-PCR–positive bats developed antibodies reactive against ebolavirus proteins from Zaire ebolavirus–infected cells (*6*). In this study, both BOMV RT-PCR–positive and RT-PCR–negative bats displayed only mild mononuclear infiltrates in lungs and exhibited mild inflammatory changes in other tissues. Those changes indicate that BOMV infection is likely well-tolerated by *M. condylurus* bats, which is consistent with expectations of a reservoir host (*12,13*). However, our findings should be interpreted with caution. For example, we cannot exclude the possibility that the BOMV RT-PCR–positive *M. condylurus* bats we captured had less severe pathology than infected wild conspecifics or that negative control bats might have been previously infected but cleared any residual viral antigens.

Positive immunolabeling with EBOV VP40 polyclonal antibody was observed in lungs from 2 of 3 BOMV RT-PCR–positive bats (Figure 2) but not in other tissues or in any tissues from BOMV RT-PCR–negative bats. Immunopositive-labeling occurred as granular cytoplasmic aggregates in interstitial macrophages. In contrast to their usual role in protective immunity, macrophages are directly
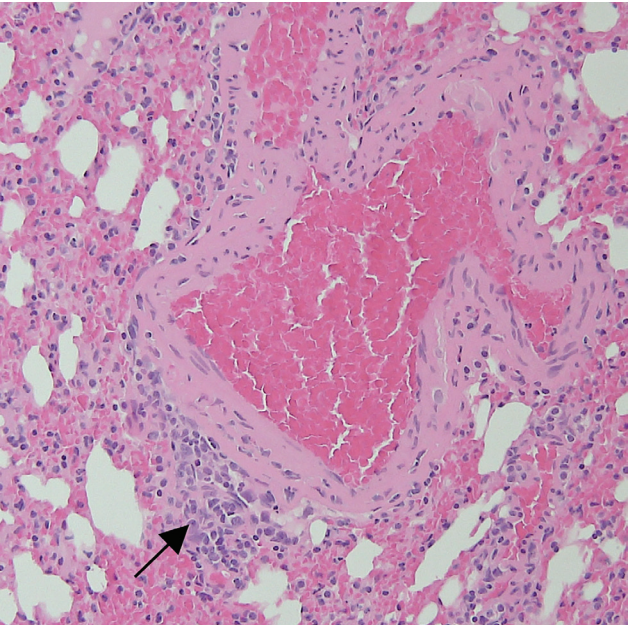
implicated in the pathogenesis of several viruses as either viral repositories or sites of active replication and dissemination (*14*). In humans, pathogenic filoviruses rely heavily on macrophages, which are major replication sites (*15*). Our findings suggest that macrophages might provide similar chronic reservoirs for BOMV infections in bats. However, without data showing active replication, we cannot rule out that our findings in macrophages have a different interpretation.

Positive immunolabeling in bat lungs was consistent with previous RT-PCR results (*8*). Lungs are the most frequent tissue infected by BOMV and have markedly higher viral loads than other tissues (*6–8*). Of note, 1 lung sample, 2 spleen samples, and 1 liver sample that were BOMV RT-PCR–positive (*8*) had no detectable EBOV VP40 antigen. Those results are most likely because immunohistochemistry has lower sensitivity than PCR, and a non–BOMV-specific antigen was used.

## Conclusions

We propose that the presence of BOMV in macrophages and absence of acute pathology typical of ebolaviruses (necrosis, thrombosis, hemorrhage, and edema) support bats as a chronic subclinical BOMV reservoir with the potential for infection and sporadic virus excretion. However, the small number of samples, absence of recovery of infectious virus, and lack of longitudinal data make interpretation of our results difficult. As for all wildlife-borne ebolaviruses, no direct evidence currently exists regarding the transmission route of BOMV between susceptible hosts. Our findings suggest the mode of transmission involves activation of replication in either macrophages or other cells and virus dispersal through saliva droplets or feces. The identification of BOMV infection in lungs and occasional virus presence in intestines further supports this hypothesis (*6–8*).

In summary, we showed EBOV-VP40 immunolabeling, acting as a cross-reactive surrogate for BOMV antigen, in pulmonary macrophages of wild BOMV RT-PCR–positive *M. condylurus* bats and an absence of substantial concurrent pathologic findings. Our results support *M. condylurus* bats as reservoir hosts for ebolaviruses and highlight the importance of bat surveillance to understand and mitigate potential emerging disease risks.
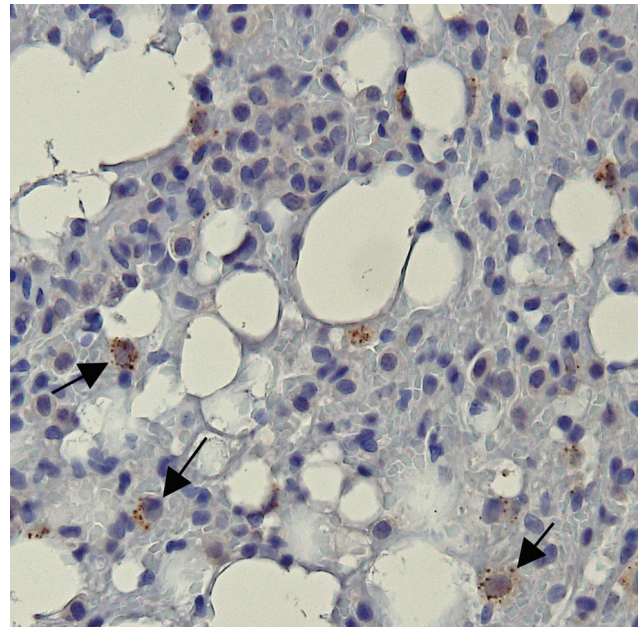
**Figure 2.** Representative bat lung tissue showing Ebola virus (EBOV) cytoplasmic granules in study of histopathologic changes in *Mops condylurus* bats naturally infected with Bombali virus, Kenya. We labeled lung tissue sections by using rabbit polyclonal serum against EBOV matrix protein VP40 and detected antigen by using a chromogenic horse radish peroxidase substrate. The sections were then counterstained with hematoxylin. Arrow indicates granular cytoplasmic immunopositivity for EBOV VP40 antigen. Original magnification ×400. VP, viral protein.
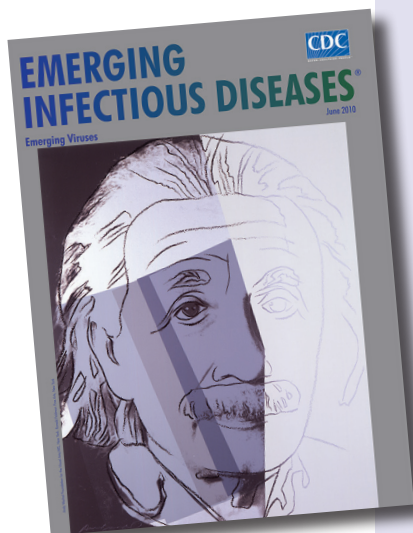
## About the Author

Dr. Kareinen is a veterinarian and PhD student at the University of Helsinki, Finland. His research interests focus on the maintenance and circulation of zoonotic viruses in bat hosts.

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# etymologia revisited

## Lassa Virus

[lah sə] virus



**Originally published in June 2010**

This virus was named after the town of Lassa at the southern end of Lake Chad in northeastern Nigeria, where the first known patient, a nurse in a mission hospital, had lived and worked when she contracted this infection in 1969. The virus was discovered as part of a plan to identify unknown viruses from Africa by collecting serum specimens from patients with fevers of unknown origin. Lassa virus, transmitted by field rats, is endemic in West Africa, where it causes up to 300,000 infections and 5,000 deaths each year.

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*https://wwwnc.cdc.gov/eid/article/16/6/et-1606_article*

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## Appendix

### IHC protocol

Antigen retrieval was performed in citrate buffer (pH 6). Tissue sections were prepared on slides and incubated for 20 min at a maximum temperature >99°C during antigen retrieval. After antigen retrieval, the sections were washed with 1× Tris-buffered saline (TBS) for 5 min and then incubated in 3% hydrogen peroxide for 10 min. Next, the sections were washed 2 × 5 min each in 1× TBS and then incubated in 10% bovine serum albumin for 20 min. Rabbit polyclonal antiserum Ag204 against Ebola virus matrix protein VP40 (*11*, main text) was diluted 1:500 in Animal Free Blocker and Diluent (Vector Laboratories, https://www.vectorlabs.com) and added to the tissue sections. Sections were incubated for 60 min with the primary antibody and then washed 2 × 5 min each in 1× TBS. For each sample, a negative control was similarly prepared; the primary antibody was replaced with a rabbit isotype control diluted at 1:500. Secondary antibody (BrightVision anti-rabbit HRP 1 step detection system) (Immunologic, http://www.immunologic.nl) was added to the sections and incubated for 30 min. The sections were washed 2 × 5 min each in 1× TBS. Bright DAB chromogen (Immunologic) was added to the sections and incubated for 6 min; the slides were then rinsed in water. Sections were counterstained with hematoxylin (Papanicolaou's solution 1a Harris hematoxylin solution, Sigma-Aldrich, https://www.sigmaaldrich.com) for 10 s and rinsed with tap water for 3 min.

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CLUSTAL O(1.2.4) multiple sequence alignment


tr|A0A0D5W8L9|A0A0D5W8L9_9MONO      MRRVILPTAPPEYMEAIYPARSNSTIARGGNSNTGFLTPESVNGDTPSNPLRPIADDTID      60
tr|A0A4D5SGV0|A0A4D5SGV0_9MONO      MRRTVIPTAPPDYTEALYPQRANSIGSTSTSTNQQYYPTDVPGGDIPSNSLRPVADDNID      60
                                    ***.::*****:* **:** *:**   : . .:*  :    :  .** *** ***:***.**

tr|A0A0D5W8L9|A0A0D5W8L9_9MONO      HASHTPGSVSSAFILEAMVNVISGPKVLMKQIPIWLPLGVADQKTYSFDSTTAAIMLASY     120
tr|A0A4D5SGV0|A0A4D5SGV0_9MONO      HTNHTPDNVSSAFILEAMVNVISGPKVLMKQIPIWLPLGVADQKKYSFDSTTAAIMLASY     120
                                    *:.***..*******************************.***************

tr|A0A0D5W8L9|A0A0D5W8L9_9MONO      TITHFGKATNPLVRVNRLGPGIPDHPLRLLRIGNQAFLQEFVLPPVQLPQYFTFDLTALK     180
tr|A0A4D5SGV0|A0A4D5SGV0_9MONO      TITHFGKTSNPLVRVNRLGPGIPDHPLRLLRLGNQAFLQEFVLPPVQLPQYFTFDLTALK     180
                                    *******::**********************.**************************

tr|A0A0D5W8L9|A0A0D5W8L9_9MONO      LITQPLPAATWTDDTPTGSNGALRPGXSXHPKLRPILLPNKSGKKGNSADLTSPEKIQAI     240
tr|A0A4D5SGV0|A0A4D5SGV0_9MONO      LITQPLPAATWTDEVLLTSPNMLRPGLSFHPKLRPILLPGKPGKKGANLGLTAPDKIHAI     240
                                    *************:.   * . **** * **********.* ****  . .**:*:**:**

tr|A0A0D5W8L9|A0A0D5W8L9_9MONO      MTSLQDFKIVPIDPTKNIMGIEVPETLVHKLTGKKVTSKNGQPIIPVLLPKYIGLDPVAP     300
tr|A0A4D5SGV0|A0A4D5SGV0_9MONO      MDHLQDLKVVPIDSSKNIVGIEVPDALVQKLTGKKPHAKNGQSIIPVLLPKYIGPDPVSA     300
                                    *  ***:*:**** :***:*****::**:******  :**** *********** ***:

tr|A0A0D5W8L9|A0A0D5W8L9_9MONO      GDLTMVITQDCDTCHSPASLPAVVEK    326
tr|A0A4D5SGV0|A0A4D5SGV0_9MONO      SDLTMVITQDCDTCNSPASFPIASEK    326
                                    .*************:****:* . **
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**Appendix Figure.** Alignment of Ebola virus (UniProt no. A0A0D5W8L9) and Bombali ebolavirus (UniProt no. A0A4D5SGV0) VP40 matrix proteins. We used the UniProt protein database (https://www.uniprot.org) align-tool and found ≈75% amino acid identity between the 2 VP40 proteins. Asterisks represent fully conserved residues between sequences, colons represent conservation between amino acids with strongly similar properties, and periods represent conservation between amino acids with weakly similar properties.